

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : DONG, Zheng Xin	Examiner : LUKTON, David
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P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JUNDONG ZHANG UNDER 37 C.F.R. §1.132

I, Jundong Zhang, Ph.D., hereby declare and state that:

1. I have a Ph.D. in chemistry and I serve as scientist at Biomeasure, Incorporated, 27 Maple Street, Milford, MA 01757-3650. It is a routine part of my job to test the half-lives of novel compounds using the assay method(s) commonly employed in the field.
2. I am familiar with the subject matter claimed in the above-identified patent application.
3. I have tested the compounds in the below Table 1 to measure their plasma half-lives, by following a commonly employed assay methodology. Details of the procedure used to evaluate the plasma stability of the example compounds are recited below:

GLP-1 peptide (50 μ L 500 μ g/mL) was added to 450 μ L plasma (human, rat, or mouse), vortexed briefly and incubated at 37°C. 50 μ L was removed at various times, like at 0, 0.5, 1, 2, 3, 4, 8, 24, 48 hours, mixed with 150 μ L acetonitrile in a microcentrifuge tube, vortexed, and centrifuged for 10 minutes at 10K rpm. The supernatant was transferred to an injection vial and analyzed by LC-MS. The LC-MS system consisted of a Finnigan Deca XP mass spectrometer with an ESI probe. Positive ion mode and full scan detection were used. HPLC separation was carried out on a

Luna 3 μ C8 (2), 3 x 50 mm column with a gradient from 100% A to 80%B in 10 minutes at a flow rate of 0.25 ml/min. Buffer A was 0.1% acetic acid in water and buffer B was 0.1% acetic acid acetonitrile.

4. The results of the plasma half-life assay for the seven example compounds are shown below as Table 1:

TABLE 1

Example No.	Structures	Rat plasma T $\frac{1}{2}$ (hours) at 37°C
2	(Aib ^{8,35} , Arg ^{26,34} , Phe ³¹ , Pro ³⁷ , Ser ^{38,39})hGLP-1(7-39)-NH ₂	11.0
3	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , Asn ³⁸)hGLP-1(7-38)-NH ₂	10.3
10	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , Ser ³⁸)hGLP-1(7-38)NH ₂	17.8
11	(Aib ^{8,35,37} , Gaba ³⁸)hGLP-1(7-38)NH ₂	16.7
12	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , His ³⁸)hGLP-1(7-38)NH ₂	14.2
13	(Aib ^{8,35} , Arg ^{26,34} , Phe ³¹ , β -Ala ³⁷ , His ³⁸)hGLP-1(7-38)NH ₂	9.7
14	(Aib ^{8,35,37} , Arg ^{26,34} , D-His ³⁸)hGLP-1(7-38)NH ₂	10.8
15	(Aib ^{8,35,37} , β -Ala ³⁸)hGLP-1(7-38)NH ₂	30.4
20	(Aib ^{8,35} , Arg ^{26,34} , β -Ala ³⁷ , His ³⁸)hGLP-1(7-38)NH ₂	12.4
21	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , Gly ³⁸)hGLP-1(7-38)NH ₂	13.2
22	(Aib ^{8,35,37} , Arg ^{26,34} , Gly ³⁸)hGLP-1(7-38)NH ₂	19.3
23	(Aib ^{8,35,37} , Arg ^{26,34} , β -Ala ³⁸)hGLP-1(7-38)NH ₂	17.7
24	(Aib ^{8,35,37} , Arg ^{26,34} , Gaba ³⁸)hGLP-1(7-38)NH ₂	23.7
25	(Aib ^{8,35,37} , Arg ³⁴ , Phe ³¹ , His ³⁸)hGLP-1(7-38)NH ₂	13.1
26	(Aib ^{8,35,37} , Arg ^{26,34} , His ³⁸)hGLP-1(7-38)NH ₂	22.4
27	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , Gaba ³⁸)hGLP-1(7-38)NH ₂	12.4
28	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , Ava ³⁸)hGLP-1(7-38)NH ₂	15.1
29	(Aib ^{8,35,37} , Arg ^{26,34} , Ava ³⁸)hGLP-1(7-38)NH ₂	21.7
30	(Aib ^{8,35,37} , Arg ³⁴ , Phe ³¹ , D-His ³⁸)hGLP-1(7-38)NH ₂	13.1
31	(Aib ^{8,35,37} , Arg ³⁴ , Phe ³¹ , Gly ³⁸)hGLP-1(7-38)NH ₂	10.4
33	(Aib ^{8,35,37} , Gly ³⁸)hGLP-1(7-38)NH ₂	12.6
34	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , D-His ³⁸)hGLP-1(7-38)NH ₂	12.4
35	(Aib ^{8,35} , Arg ^{26,34} , Phe ³¹ , β -Ala ³⁷ , D-His ³⁸)hGLP-1(7-38)NH ₂	9.2
36	(Aib ^{8,35,37} , Arg ^{26,34} , Phe ³¹ , β -Ala ³⁸)hGLP-1(7-38)NH ₂	10.4

37	(Aib ^{8,35} , Arg ^{26,34} , Phe ³¹ , β -Ala ^{37,38})hGLP-1(7-38)NH ₂	13.2
38	(Aib ^{8,35,37} , Arg ³⁴ , Phe ³¹ , β -Ala ³⁸)hGLP-1(7-38)NH ₂	17.8
39	(Aib ^{8,35,37} , Arg ³⁴ , Phe ³¹ , Gaba ³⁸)hGLP-1(7-38)NH ₂	12.2

5. I declare that all statements made herein of my own knowledge are true and that statements made upon information and belief are believed to be true, and further that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 10/22/2010


Jundong Zhang, Ph.D.